

Transpiration measurements on single leaves from mite-infested orange plants involve determination of water loss from leaves with petioles sealed in a vial of water. Because the weight loss is continuous but very small, weighings must be made rapidly on an extremely sensitive balance. Mites may be seen feeding on the leaf being weighed.

Attempts to measure the effects of mite damage on various physiological processes in citrus plants—particularly the leaves—in field, greenhouse and laboratory experiments have added to the relatively little available information concerning the effects of red mite feeding.

Leaf stippling, or scarring, is one of several deleterious effects—such as excessive leaf drop, twig dieback, reduced juice quality and decreased yield—associated with red mite infestation when there is no control program or the trees are in a weakened condition from other causes.

The results of the experiments endeavoring to measure the effects of mite feeding were indicative rather than conclusive, but, when better procedures become available with modern developments in instrumentation, definitive observations can be taken.

24''—Eureka Small-approximately lemon plants grown from rooted cuttings were used in greenhouse experiments for measurements of photosynthesis and respiration. Mite damage was assured by infesting the plants with about 35 mites per leaf. Two weeks later-when considerable stippling of the leaves had become apparent-the mites were washed from the leaves with a forceful spray of fine jets of water. Several days later comparative measurements of photosynthesis and respiration were started-and continued for more than six months-on mite-damaged leaves and on leaves from uninfested check plants.

The rates of photosynthesis of mitedamaged leaves were calculated—on the basis of microliters of oxygen produced per square centimeter of leaf surface per

# experiments designed to measure damage give bases for further studies

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hour-as percentages of the rates of the untreated leaves at the various time intervals. On the first sampling date the damaged leaves had a rate of photosynthesis appreciably lower than normal leaves. The difference was statistically significant at the 5% level, and the lower rate in the damaged leaves continued to be significant at this level-with one exception-until 26 weeks after the mites were first applied. At that time the relative photosynthesis of the stippled leaves was at a level slightly, but not significantly, higher than the controls. Part of the considerable variability in the measurements may be because not all leaves were equally damaged by the mites, nor were all the leaves of uniform age. In spite of this, it is clear that for several months the damaged leaves produce carbohydrates at a rate lower than normal leaves. After that time the inhibition due to previous mite damage could no longer be demonstrated.

Respiration measurements — at the time of the photosynthesis determinations—indicated no significant effect of mite damage, which may not be conclusive because the methods used did not

Effect of mite damage on photosynthesis and transpiration of Eureka lemon plants. Leaves were damaged and mites removed before first measurements were made.



provide optimum conditions for measurement of respiration.

Additional experiments, in which the photosynthesis of mite-damaged leaves was compared with that of normal leaves, provided further confirmation of the inhibitory effect of mite feeding. For example, leaves collected in March in Orange County-from Eureka lemon trees which had not received any pesticide treatment-had a rate of photosynthesis only 84% that of leaves from adjacent trees which had been treated with oil spray in September and with ovex in April. The leaves from the untreated trees had an average of 65 stipple marks per mm<sup>2</sup>—per square millimeter— while leaves from the treated trees had an average of only 10 stipple marks per mm<sup>2</sup>. However, at other times of the year, leaves from the same trees with about the same extent of mite damage did not show any inhibition of photosynthesis.

In another experiment, leaves of Bearss lime plants growing in the greenhouse—which were damaged by mites to the extent of 92 stipple marks per mm<sup>3</sup>—had a rate of photosynthesis only 79% that of similar undamaged leaves.

The loss of water through leaves is likely to be influenced by mite feeding activities. Some of the observed effects of heavy mite infestations on citrus such as leaf drop—could be attributed to severe alterations in transpiration. Accordingly, several experiments were conducted to evaluate the effects of mite damage on the transpiration of citrus.

In one greenhouse experiment, the transpiration of young Éureka lemon plants-similar to those used for the photosynthesis experiment — was followed for more than six months after the leaves had been damaged by mite feeding. Mites were allowed to feed on the leaves of one group of plants until there was an average of 120 stipple marks per mm<sup>2</sup> of leaf surface. A second group of plants was maintained free of mites and served as a control. To insure that transpiring leaves were mite damaged, the leaves of the older growth flush which were not attacked by mites were removed prior to the start of transpiration measurements. The leaves of the same flush were also removed from the untreated plants. Six weeks were required Continued on next page

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to bring all the plants to the desired state of mite damage. At the end of that period all mites were removed by washing the leaves, and the plants were kept free of mites thereafter by washing the leaves twice weekly.

Transpiration was measured by enclosing the pot in a polyethylene bag which was gathered and tied around the stem of the plant to prevent any water loss except through the leaves. The individual plants were then weighed on a sensitive balance in the morning, and after a period of about six hours in the greenhouse were again weighed to determine the weight loss. At the end of the experiment the total leaf area was measured with a photoelectric arealimiter. The area of any leaf dropped during the experiment was determined at once and was used for computation of transpiration rates only during the period the leaf was attached to the plant.

The transpiration rates were calculated as milligrams of water loss per square centimeter of leaf surface per hour, but —for convenience in comparison with the transpiration of the mite-damaged plants—the rates are represented in the graph on the preceding page as a percent of that of the untreated plants.

The effect of mite feeding on transpiration of lemon plants was similar to the responses obtained in the photosynthesis measurements—an initial inhibition of water loss from the damaged leaves disappeared after about five months. The initial differences were significant in most cases at the 1% level and the significant inhibition persisted until the thirtieth week.

In another experiment, the effect of mite feeding on the transpiration of small Algerian tangerine and Marsh seedless grapefruit was determined. The plants were similar to those in the lemon experiment except that they were grafted on lemon rootstocks. The treatment and measuring procedures were similar, but mites were placed on the treated plants only 10 days prior to the start of measurements. Additional mites were deposited on the leaves two or three times a week during the four-week span of the experiment-to maintain the infestation -because the plants were kept at a relatively high temperature with a low humidity and the mite population did not build.

The results indicated that mite feeding under the conditions of the experiment had no effect on the transpiration of tangerines, but the process in grapefruit plants was depressed significantly for at least a month.

Mite feeding on citrus leaves injures a number of cells in-and below-the epidermis and perforates the protective cuticle. Therefore, the finding that mitedamaged leaves lost water at a slower rate than normal leaves was unexpected.

Because these experiments involved damage resulting from the presence of mites from several days to several weeks —prior to the initiation of measurements—a more sensitive technique was used in additional experiments and measurements were started at the time the mites were first placed on the plants.





In these additional experiments, 18''-20'' rooted cuttings of Washington navel orange, in 6'' pots, were placed in a temperature controlled room at  $75^{\circ}$ F and provided with artificial light. Mites were put on the leaves to provide an initial population of about 100 mites per leaf and transpiration measurements were started the same day.

Measurements were made by removing leaves from infested and control plants, sealing their petioles in vials of water, and following the water loss by periodic weighings with an analytical balance. At the end of each day the leaf areas were determined, and a new set used the following day. The transpira-

Effect of	f Mite	Feeding	Injury	on Tr	ranspire	ition	of
Tangeri	ne an	d Grape	fruit P	lants	under	Gree	en-
		house	Conditi	ions			

	Weeks after infes-	Transp (Milligran centimet	plants %	
tation		Control		
Tan-	2	4.54	5.04	111.0
gerine	6	6.55	6.20	94.7
Grape-	2	6.26	5.96*	95.2
fruit	6	8.29	6.24**	75.3

\*, \*\* Significantly lower than undamaged plants at 5% and 1% levels of significance. tion rates were computed as milligrams per square centimeter per hour, but are represented in the graph on this page as percents of the control leaf rates.

The mite feeding first produced a considerable stimulation of transpiration of the orange leaves, reaching a maximum on the second day after feeding started. However, the stimulation quickly declined until at the end of a week—as in the previous experiments—there was an appreciable inhibition of transpiration in the damaged leaves.

The strong increase in water loss found shortly after the start of mite feeding may result-indirectly-in the prolonged inhibition which follows. The severe stress brought about by the very rapid initial increase in transpiration may cause injury to the water conduction system-perhaps by formation of callose plugs in the vessels of stems and leavesand may also affect the operation of the guard cells which open and close the stomatal pores through which most of the water loss takes place. Injured guard cells-resulting from excessive water loss-might become inoperative and reduce transpiration by remaining closed. Feeding of mites-by direct removal of the cell contents or by injury to adjacent cells-may injure the guard cells. Most of the stomates in citrus are located on the lower surface of the leaf, where less injury from mite feeding is usually apparent, but in heavily stippled leaves it is likely that the mites feed near a sizeable percentage of the stomates.

Permanent damage to the water conduction system or the stomates may be involved because recovery from the inhibitory effects of mite feeding on the leaves of lemon plants in the earlier transpiration experiments was primarily due to a decrease in the transpiration of the undamaged leaves toward the end of the experiment rather than to an increase in the transpiration of the mitedamaged leaves. The aging control leaves may have reached a condition similar to that in the experimental leaves that was caused initially by the mite feeding.

A similar situation—the apparent recovery in photosynthesis by damaged leaves—was actually more due to a gradual decrease in the rate of photosynthesis of the control leaves with time.

Because both photosynthesis and transpiration appear to respond in the same way the effects of mite feeding may be due to some malfunction of the stomatal apparatus as failure of the guard cells to open would tend to reduce both photosynthesis and transpiration.

Measurements of juice quality factors —such as percent acid, total soluble solids, percent juice—in Eureka lemons in Orange County were made during a period of more than a year. They re-Concluded on page 12

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ferent when fertilizers were placed 4" under the seed instead of 2". Doubling the rate of application—to 40 pounds of nitrogen and 50 pounds of phosphorus pentoxide per acre—definitely increased the growth of the plants, but the effect generally disappeared shortly after thinning.

Accurate fruit yield records were difficult to obtain in 1957. Extremely hot weather in June and July delayed fruit set, which may have reduced the earlier maturity observed in previous years.

Plant Weights at Thinning Time, 1957

Treatment				Ave. plant wat.			
Pounds/acre		Place-	Grams				
N <sup>1</sup>	P2052	<b>K</b> <sub>2</sub> <b>O</b> <sup>3</sup>	ment below seed	Davis	Tracy	Stock- ton	
0	0	0		2.08	0.65	0.91	
0	25	0	2"		2.42		
20	25	0	2″	7.78	2.72	3.00	
20	25	25	2"		2.90	3.58	
20	25	0	4"	7.96	2.71	3.09	
40	50	0	2″	8.81	2.89	3.53	
	LSD4 5	%		1.04	0.53	0.43	
1	Nitrog	8	Phasehos			8 Deter	

sium oxide. 4 Least significant difference.

Furthermore unseasonable rains cut short the harvest season, and in most plots several tons of tomatoes per acre were left in the field. Yield records of one plot were not taken, because of nematode infestation. The yield results in which some confidence may be placed are shown in the larger table on this page. Generally, close placement of fertilizer under the seed—either phosphorus or phosphorus plus nitrogen—produced greater early yields of fruit, but total yields were not greatly affected.

In one trial in San Joaquin County on Staten Island—liquid 8-24-0 was used instead of the dry fertilizer used in the under-seed fertilizer treatment. Results from this test were quite comparable to the others in the effect of fertilizer on both plant growth and early yield. Certain inherent dangers exist with these fertilizer practices. Close placement may, under certain conditions, allow soluble salts to migrate into the seed band and interfere with germination. However, proper irrigation and fertilizer band placement should avoid damage by soluble salts. Anhydrous or aqua ammonia should not be used in this manner because each one is very toxic—when placed too close to plants—and can inhibit germination.

The main effect of close placement of fertilizer may be an indirect one. The fertilizer stimulates growth to such an extent that fertilized plants are much larger when environmental conditions permit fruit set to begin. This provides a greater number of flowers that set at one time, which results in greater early yields.

Some indirect benefits are also apparent. Close placement stimulates a more vigorous root system, which allows the plant to explore the soil mass for other needed nutrients. Faster growth of the seedling considerably simplifies the weed control problem, since closer cultivation can be practiced at an earlier date. It also provides a shorter period of susceptibility to such seedling insect pests as flea beetles.

The results of the placement trialscoupled with previous fertilizer trialscan be translated into generalized fertilizer treatments for this crop. Treatments of 10-20 pounds per acre of nitrogen and 25 pounds of phosphate-phosphorus pentoxide—placed 2"-4" directly beneath the seed should be applied at planting time. About 60 pounds of nitrogen in the ammoniacal form, should be applied as a sidedressing shortly after thinning. The small amount of nitrogen applied at planting is probably not sufficient for maximum yields, and larger amounts might lead to germination troubles or be leached from the soil before the plants can use it.

Similar trials—continued in 1958 are designed to study the effect of these fertilizer practices on direct-seeded toma-

Tomato Fertilizer Yields, 1957 Tests

Treatment			Yields, tons/acre					
Pound/acre			Woodland		Stockton		Tracy	
Nı	P2052	K <sub>2</sub> O <sup>3</sup>	Placement	1st harvest	Total harvest	l st harvest	Total harvest	lst harvest
0	0	0	Sidedress	3.3	14.2	8.5	21.3	9.7
60	0	0	14	3.3	15.7			
120	ō	0	"	2.6	14.4	7.9	20.6	
120	120	0	"	3.9	16.8			
120	120	120	"	5.3	18.3			
0	25	0	2" Under seed					9.0
20	25	Ó	"	4.3	19.7	11.8	22.1	
20	25	25	"					10.9
40	50	0	"					10.0
20	25	0+	"	7.0	19.8	9.3	21.2	
.60	0	ŏ	Sidedress					
	LSD4 5%			3.06	2.60	2.40	N.5.	N.S.

<sup>1</sup> Nitrogen. <sup>2</sup> Phosphorus pentoxide. <sup>8</sup> Potassium oxide. <sup>4</sup> Least significant difference.

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The above progress report is based on Research Project No. 1129

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vealed no significant differences—as a result of mite infestation—when untreated, mite-damaged trees were compared with trees treated with oil sprays and acaricides. Neither was there any significant effect of mite damage on the production of trees receiving no pest control measures as compared with treated trees. In a few cases mite infested trees yielded less fruit at one pick than the best of the pest control treatments, but the differences were not statistically significant.

The Orange County trees were young and vigorous with a relatively light crop in proportion to total leaf area. There were several periods during the experiments when the leaves of a single growth flush were noticeably injured by mites; but other growth flushes in the same year were not as severely injured. The vigor of the trees, the relatively small ratio of fruit to foliage, and the breaks in the continuity of mite damage, may have been factors in the lack of larger differences in crop.

Certain special conditions of the experimental plots may have helped protect the untreated trees from the full effects of mite infestation. The plots were small —four trees in a square—and the treatments consisted of an application timing series. Such conditions would be favorable to populations of mite predators, while the over-all population of citrus red mites in the plot would be knocked down by the spray treatments.

In similar experiments with navel orange trees at Corona, the check trees were severely infested with citrus red mite during the fall of 1957 and there was a heavy leaf drop. Although at the time of harvest the total number of fruit was not reduced, the buttons were discolored and the fruit was soft, resulting in lowering of the grade to culls.

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